Evaluating Extracted Chromatin From Fixed Mice Organ Tissues

Lauren Sweeney[1], Michelle D. Thomas[1], Daphnie Velasquez Garcia[1], Marjan Mehrab-Mohseni[2], Brian Velasco[2], Paul A. Dayton[2,3], Ian J Davis[3,4,5], and Samantha G. Pattenden[1,3]

[1]Division of Chemical Biology and Medicinal Chemistry, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill;[2]Joint Department of Biomedical Engineering, University of North Carolina at Chapel Hill;[3]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill; [4]Department of Genetics, School of Medicine, University of North Carolina at Chapel Hill;[5]Division of Pediatric Hematology-Oncology, Department of Pediatrics, School of Medicine, University of North Carolina at Chapel Hill.

Abstract

Nucleosomes consist of DNA wrapped around histone proteins. Together the DNA, histones, and other associated proteins are called chromatin. Nucleosome positioning along the DNA affects the ability of transcriptional proteins to access the DNA and therefore affects DNA expression. This is called chromatin accessibility (nucleosome rich areas are said to be inaccessible and nucleosome poor areas are accessible). We have developed a first-in-class nanodroplet-mediated formaldehyde-assisted isolation of regulatory elements (FAIRE) assay to extract DNA and associated proteins from formalin-fixed paraffin-embedded (FFPE) tissues so it can be used in future chromatin accessibility assays. Because FFPE tissue processing is not standardized, this study works to understand the effect of storage condition time at 4° Celsius (for 0 hours, 4 hours, and 24 hours) before fixation on extracted chromatin quality from male C56BL6J mouse liver and kidney organs using previously developed FAIRE Evaluation Metrics (FEM). As storage time increases, we expected a decrease in chromatin quality. We found that as storage condition time increased, there was an increase in percent soluble chromatin, a lack of detectable DNA fragments, and a decreasing signal over background in enrichment for accessible chromatin via qPCR. As Biobanks increase FFPE tissue storage, there is a need for technologies to extract high-quality chromatin from preserved samples.

Chromatin Extraction From FFPE Mouse Organ Tissue

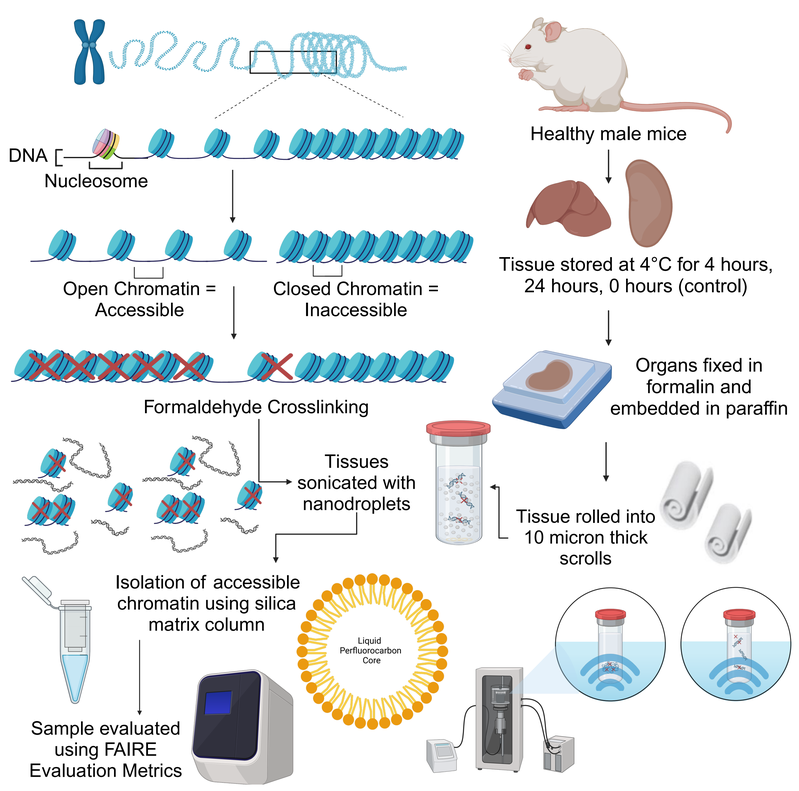

Figure 1. High-quality chromatin is extracted from mice FFPE organ tissues using a nanodroplet-mediated formaldehyde-assisted isolation of regulatory elements (FAIRE) assay.

FAIRE Evaluation Metrics (FEM)

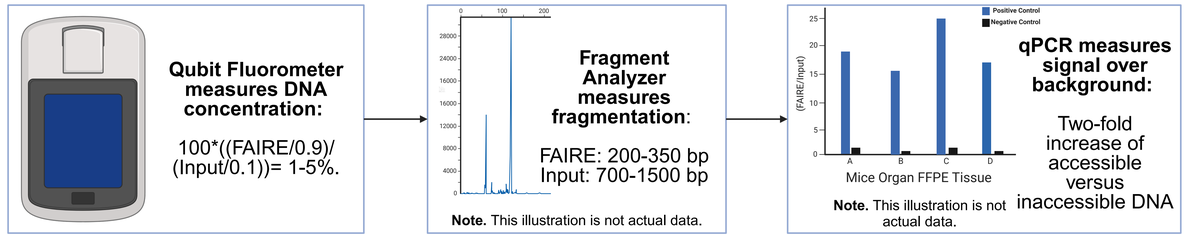

Figure 2. This study uses three FAIRE evaluation metrics (FEM) to understand the effect of storage condition times on extracted chromatin.

Evaluation of Extracted Accessible Chromatin

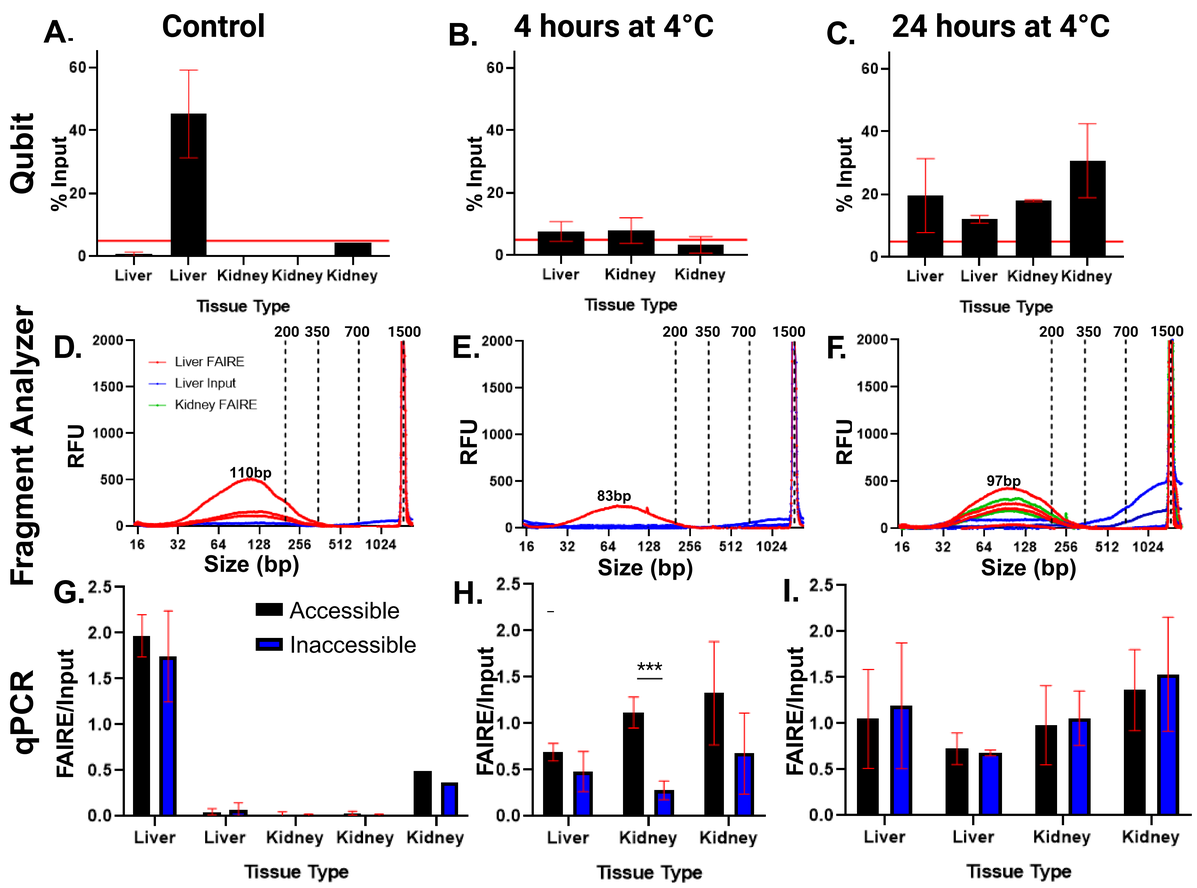

Figure 3: Qubit Fluorometer concentrations were used to calculate the percent of input (percent of soluble chromatin) shown on the y-axis with A. 0 hours B. 4 hours and C. 24 hours at 4°C. The Fragment Analyzer was used to measure the relative fluorescence units (RGU) of specified base pair lengths D. 0 hours E. 4 hours F. 24 hours at 4°C. qPCR was used to measure signal (positive or accessible regions of chromatin) over background (negative or inaccessible regions of chromatin) in G. 0 hours H. 4 hours I. 24 hours at 4°C with the y-axis representing percent input.

Conclusion and Future Directions

- Chromatin was successfully extracted from liver and kidney tissues
- Extracted chromatin quality and quantity varied across storage condition time
- Because of control sample inconsistencies, no further conclusions can be drawn
- Possible reasons for inconsistencies include over or under-sonication, and inconsistent tissue size
- Future directions include altering sonication time (more sonication for larger tissue pieces) and to develop primers specific to accessible and inaccessible regions in desired organs

Acknowledgements

Funding for this work was provided by NIH/NIGMS R01GM138912 (SGP and IJD).

Disclosures: S.G.P., I.J.D., and P.A.D. own equity in Triangle Biotechnology, Inc. S.G.P and P.A.D. are inventors on intellectual property related to this research. This IP is licensed to Triangle Biotechnology, Inc. from University of North Carolina at Chapel Hill.

Marcel, S. S., Quimby, A. L., Noel, M. P., Jaimes, O. C., Mehrab-Mohseni, M., Ashur, S. A., Velasco, B., Tsuruta, J. K., Kasoji, S. K., Santos, C. M., Dayton, P. A., Parker, J. S., Davis, I. J., & Pattenden, S. G. (2021). Genome-wide cancer-specific chromatin accessibility patterns derived from archival processed xenograft tumors. Genome research, 31(12), 2327–2339. https://doi.org/10.1101/gr.275219.121